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Body mass index and glucose variations during the night in free-ranging *Artibeus planirostris* (Chiroptera: Phyllostomidae)

Luane Maria Melo Azeredo[1], Monique Silva Ximenes[2], Kleytone Alves Pereira[3], Maria Paula Aguiar Fracasso[4], Luiz Carlos Serramo Lopez[1]

[1]*Laboratório de Ecologia Comportamental e Psicobiologia, Departamento de Sistemática e Ecologia, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba. Campus I, Cidade Universitária, Castelo Branco, 58051-900 João Pessoa, PB, Brazil.*
[2]*Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental, Universidade Federal da Paraíba. Campus IV, Rio Tinto, 58297-000 PB, Brazil.*
[3]*Programa de Pós-Graduação em Psicobiologia, Universidade Federal do Rio Grande do Norte. Campus Lagoa Nova, 59078-970 Natal, RN, Brazil.*
[4]*Centro Universitário de João Pessoa. Rodovia BR 230, km 22, Água Fria, 58053-000 João Pessoa, PB, Brazil.*
Corresponding author: Luane Maria Melo Azeredo (luaneazeredo@gmail.com)

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ABSTRACT. Body condition is an important measure to estimate the energy reserve of an organism. Scientists frequently use body condition indices (BCIs) with morphometric measures but direct measurements, such as blood glucose, seem to be more reliable. We observed oscillations in the body condition and glucose indexes of individuals of *Artibeus planirostris* (Spix, 1823) during 13 nights in the field. We assume that if glucose levels are proportional to feeding state and body condition is a measure of energy reserve, blood glucose and BCI should be positively correlated and both are expected to increase during the night as the bats leave their diurnal roost to feed. To test this, we examined the relationship between blood glucose levels, BCI and reproductive phase of free flying male bats (n = 70) for 12 hours after sunset for 13 nights. Bats were captured in Reserva Biológica de Guaribas (Paraíba, Brazil) using mist nets. Blood glucose was analyzed with a portable glucometer. Supporting our assumptions, the number of hours after sunset and BCI presented significant positive correlations with glucose levels in *A. planirostris*. Reproductive phase did not present a significant correlation with any other variables. As we predicted, glucose level can be used as proxy for morphometric BCI and it can be measured with a simple portable glucometer. The increase both in glucose and BCI around the night can be explained by the efficient assimilation of nutrients present in fruits ingested by bats and the quick metabolism that increases the levels of glucose (an other nutrients) in blood, increasing the body mass.

KEY WORDS. Body condition, blood metabolites, foraging.

INTRODUCTION

Body condition is an important ecological attribute that provides an estimate of the energy reserve of an organism (Speakman 1997, Stevenson and Woods 2006, Labocha and Hayes 2012). Moreover, this measure may have an important role in estimating the reproductive success and survival rate of animals (Moya-Larano et al. 2008). Body condition can be estimated using morphometric body condition indices (BCIs). These indexes are based on the relationship between body mass and linear body measurements. The basic premise of this index is that if individuals in a population have similar biometric measurements, for instance length of forearm, the difference between their body masses would represent different measures of their energy reserves (Speakman and Racey 1986, Speakman 2001).

However, these BCI's need to be carefully analyzed and associated with other methods of estimating body condition, to avoid misinterpretations (Waye and Mason 2008). Besides that, morphometric BCIs may be not sensitive enough to detect variations in the body condition of animals in habitats that have a constant food supply, since there is no need to store fat for periods when resources are scarce (Jenni-Eiermann 1998). In

addition to morphometry, measurements of internal nutrient levels, like the ones estimated from blood samples analysis, are more direct and can be used to determine the physical condition of individuals (Stevenson and Woods 2006, Wilder et al. 2016). These physiological data include hematocrit, triglycerides, glucose and ketones estimations, which results can be a proxy for health and feeding state (Mcguire et al. 2009, Morais et al. 2014, Azeredo et al. 2016). Besides being more direct, blood chemistry measures can be used for all individuals in a population (Stevenson and Woods 2006). A disadvantage of using only BCI is that it may not reliably infer body condition of some individuals, such as pregnant females for instance, due to the influence of the fetal body mass (Speakman 2001, Stevenson and Woods 2006).

Blood glucose is a metabolite that is directly correlated to feeding state (Jenni-Eiermann and Jenni 1997). Bats, can be good biological models to study the relationship between glucose and body condition because they expend a considerable amount of energy by moving through active flight. Therefore, they need specific physiological adaptations that facilitate locomotion fueled by a limited reserve like lipids and glycogen (Amitai et al. 2010).

Frugivorous bats consume carbohydrate and lipid-rich fruits and the digestion occurs immediately in an efficient way (Protzek et al. 2010, Batista et al. 2016). Therefore we assume that blood glucose will be positively correlated with body condition, since glucose is the first primary source of energy for bats and they can use this resource effectively. Many studies have evaluated the daily and seasonal body mass of mammals and also have associated the body weight variation with feeding state. Handley et al. (1991) stated that the frugivore *Artibeus jamaicensis* Leach, 1821 can assimilate approximately 93% of soluble carbohydrates present in fruits of its diet. The glucose ingested is mostly oxidized and used to convert carbohydrate in fat (McNab 1976). Therefore, the ideal body condition of a bat must be a balance between its short and long-term energy requirements with energy expenditure during the flight searching for food source (Laska 1990, Altringham 1996). As the vast majority of the bats do not feed during the day, we expected that our study species would consume their energy reserves during the day and restore them during the night hours while they are active.

The studied species was the flat-faced fruit-eating bat, *Artibeus planirostris* (Spix, 1823). This species is relatively large, compared with other neotropical bats such as *Carollia perspicillata* (Linnaeus, 1758) and *Sturnira liLium* (Geoffroy, 1810) with forearm length ranging from 62 to 73 mm and body mass ranging from 40 to 69 g (Hollis 2005). It is quite generalist in terms of habitat, occupying urban spaces, mainly tropical forests, savannas, open shrub areas with grasslands, and dry forests (Ballesteros and Racero-Casarrubia 2012). Artibeus planirostris is a frugivore, and thus acts as seed disperser (Oliveira and Lemes 2010). Due to the homoeothermic condition of bats and their relatively small body size when compared to other mammals, *A. planirostris* has a high metabolic rate, so it is possible to observe oscillations in its body condition and glucose based on its feeding state along the night. There are several studies on the species of *Artibeus*, but this is the first one to investigate the relationship between body condition and blood glucose during the night under natural conditions.

MATERIAL AND METHODS

This study was conducted at Reserva Biológica de Guaribas (Rebio Guaribas), a protected area spanning part of the municipalities of Mamanguape and Rio Tinto, in state of Paraíba, Brazil (6°44'02"S, 35°10'32"W and 6°40'53"S, 35°09'59"W) The climate in Rebio Guaribas is warm and humid with an average annual temperature of 26 °C (Alvares et al. 2013). The rainy season occurs from March to August and the dry season from September to February. The seasonality was estimated by the annual precipitation data of the city of Mamanguape from the last five years obtained from the website Proclima (CPTEC/INPE 2018).

Bats were caught with nine mist nets of 12 x 3 m in two week interval expeditions of three days each from June to November of 2012, totaling 13 days. Adult animals were identified based on the absence of the epiphyseal cartilage of the 4th finger, at the metacarpal – phalangeal junction (Kunz and Anthony 1982). Individuals were caught and released on the same night. The mist nets remained open for 12 hours straight (from 5PM to 5AM) and we checked the nets every 30 minutes. Walking to the laboratory to transport the bats in containment bags took approximately 15 minutes. All individuals were marked with plastic necklaces with color cylinders (Esbérard and Daemon 1999). Our sampling effort was calculated according to Straube and Bianconi (2002).

We collected blood samples from the femoral vein using disposable calibrated lancets GTech® inserted into a lancing device GTech®. The concentrations of blood glucose were measured using reagent test strips (Freestyle®) and a glucometer Optium™ Xceed® medsense in a similar procedure of Azeredo et al. (2016). Every sample collected belonged to different bats, since we had no recaptures. Permits to capture and handle the animals were duly provided by ICMBio (license #25891–3).

We calculated the body condition index (BCI) to assess the body condition of all individuals of *A. planirostris*. We evaluated the index using Le Cren's relative condition (Kn) (Le Cren 1951). The expected body mass was assessed using residuals of an ordinary least square (OLS) regression between body mass and the forearm length of *A. planirostris*. Due to the difficulty to identify pregnant females and the potential noise in the BCI caused by the fetal body mass we used only male bats in our analysis. We classified males as non-reproductive (with abdominal testis) or reproductive (scrotal testis) according to Zortéa (2003). Data were normalized by square root transformation before being analyzed. We performed a stepwise model selection using the Akaike's information criterion to choose the model that best fitted the relationship between blood glucose and the

other variables (hours after sunset, BCI, rainy and dry seasons and reproductive phase). The best model was employed in a general linear model. We also performed a correlation matrix to observe the correlation among all continuous variables. We adopted confidence interval of 95% ($p < 0.05$) and the analyses were performed with R (R Core Team 2017).

RESULTS

The sampling effort was 37,908h*m^2. A total of 70 male bats were captured. The model that best fitted the relationship between our collected variables was that one that included glucose, BCI and hours after sunset (AIC = 205.51). Reproductive phase and the seasons (rainy and dry) were excluded by stepwise model selection and were considered non-significant. Glucose levels ranged from 1.11mmol/L to 20.36 mmol/L, (mean 7.50 ± SD 4.85), body mass ranged from 31.88 to 52.51 g (mean 43.28 ± 4.52) and forearm length ranged from 55.4 to 63.0 mm (mean 59.41 ± 1.81). The linear model showed that BCI and the hours after sunset influenced positively and significantly the levels of blood glucose (F-statistic: 16.03 on 2 and 67 DF, p-value: 2.051e-06). (see Table 1 for details). Blood glucose increased along the night so did the BCI. The correlation matrix with the selected variables is described in Table 2 and Figs 1 and 2 show the relationship between glucose and hours after sunset and BCI and hours after sunset. Table 3 shows in details how the data were organized before the analysis were performed.

Table 1. Summary of linear model results, showing the relationship between glucose concentration and the variables selected by AIC stepwise model selection, for *Artibeus planirostris* in Rebio Guaribas, PB.

Coefficients	Estimate	Std Error	t-value	p-value
Intercept	-4.08	4.89	-1.98	0.03
Hours after sunset	0.57	0.16	3.86	<0.001
BCI	5.29	1.98	2.67	<0.01

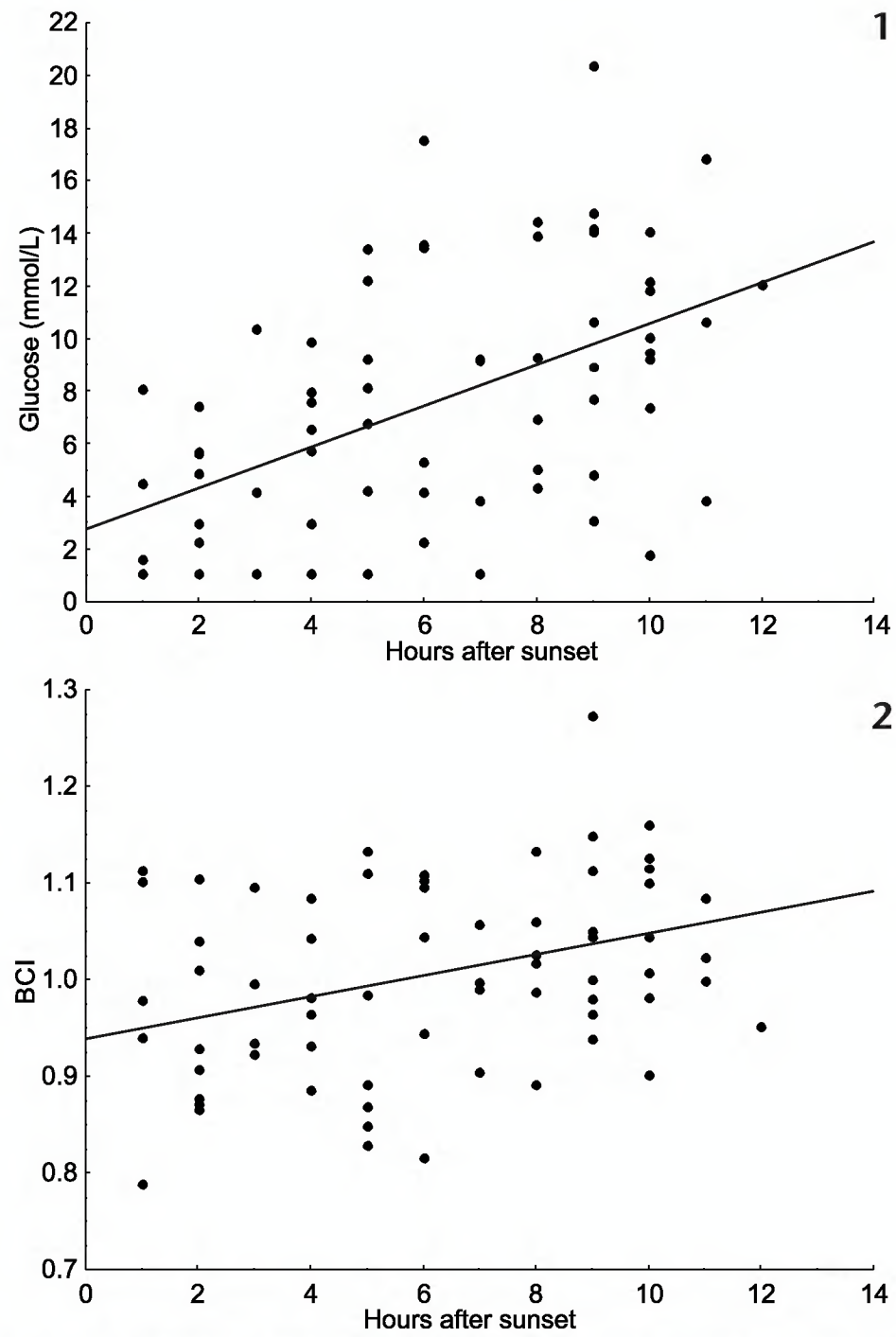
Table 2. Correlation matrix between glucose, BCI and hours after sunset for *Artibeus planirostris* in Rebio Guaribas, PB.

Variables	Glucose	Hours after sunset
Hours after sunset	0.51***	
BCI	0.41**	0.33*

*p < 0.01, **p < 0.001, ***p < 0.0001.

DISCUSSION

As predicted, glucose level was positively correlated with BCI and both variables increased their values along the night. The levels of blood glucose increased steadily after sunset, even with the bats flying and consequently expending energy. Our results were similar to Peng et al. (2017) who simulated natural



Figures 1–2. Correlation between (1) Glucose (mmol/L) and (2) BCI and the hours after sunset for individuals of *Artibeus planirostris* (N = 70) in Reserva Biológica de Guaribas, PB.

conditions in captive frugivorous bats in Southwestern China and observed that although bats expended lots of energy flying, their glucose levels increased over time (a result that could be explained because they had been fed every 15 minutes during the experiment, thus keeping their homeostasis). Therefore, we assume that the positive correlation between blood glucose and the hours after sunset for *A. planirostris* is the result of the foraging habits of bats and the rapid assimilation of carbohydrates ingested as a way to keep their body condition (Handley et al. 1991). Consequently, in the first hours the levels of glucose were lower probably because bats were still leaving their roosts after a period of fasting during the day. During this fasting period the main fuel source probably was the glycogen stored in their livers that is used to regulated the homeostasis like was described for the congeneric species *A. jamaicensis* (McNab 1976).

Some studies pointed that high levels of glucose can have detrimental effects on the health of mammals (Brownlee 2001,

Table 3. Data collected to evaluate the body condition of individuals of *Artibeus planirostris* from Jun to November of 2012 in Reserva Biológica de Guaribas, PB.

Rep. Phase	Body mass	Forearm length	Glucose (mmol/L)	Month	Season	BCI	Hours after sunset
NR	42.98	60.5	1.6095	Jun	rainy	0.979179	1
R	38.47	61.2	4.884	Jun	rainy	0.865873	2
NR	46.36	58	2.2755	Jun	rainy	1.104203	2
NR	43.13	57.2	1.11	Jun	rainy	1.042414	4
R	50.31	61.1	6.771	Jun	rainy	1.134317	5
NR	45.71	56.8	14.763	Jun	rainy	1.112969	9
R	49.96	61.1	7.3815	Jun	rainy	1.126426	10
R	45.7	57.4	4.4955	Jun	rainy	1.100474	1
NR	44.53	55.4	8.0475	Jun	rainy	1.113125	1
NR	45.01	61.4	5.661	Jun	rainy	1.009597	2
NR	39.54	58.4	1.11	Jun	rainy	0.934969	3
NR	43.38	60.9	7.9365	Jun	rainy	0.981454	4
NR	48.01	59.7	13.431	Jun	rainy	1.109222	5
NR	43.99	58.2	5.328	Jun	rainy	1.043961	6
R	42.02	58.2	3.8295	Jun	rainy	0.997209	7
NR	44.27	60	4.329	Jun	rainy	1.017425	8
NR	47.09	58.3	12.1545	Jun	rainy	1.11551	10
NR	37.06	55.6	1.11	Jun	rainy	0.922886	3
NR	41.85	61.8	2.997	Jun	rainy	0.932316	4
NR	38.12	59	1.11	Jun	rainy	0.891736	5
R	43.5	60.2	4.1625	Jun	rainy	0.99623	3
R	47.6	60.5	7.548	Jun	rainy	1.084433	4
NR	42.8	61.1	5.7165	Jun	rainy	0.964993	4
R	38.87	56.9	4.1625	Jun	rainy	0.944673	6
NR	49.75	60.5	9.324	Jun	rainy	1.133415	8
R	50.61	60.1	9.4905	Jun	rainy	1.161094	10
NR	42.69	59	16.8165	Jun	rainy	0.998642	11
NR	39	59.3	1.11	Jul	rainy	0.90746	2
NR	40.98	57.2	1.11	Jul	rainy	0.99045	7
NR	44.18	58.4	3.108	Jul	rainy	1.044687	9
R	41.23	58	9.879	Aug	rainy	0.982017	4
R	43.56	61	9.2685	Aug	rainy	0.983824	5
R	49.82	61.9	17.538	Aug	rainy	1.107979	6
NR	47.99	62.3	14.43	Aug	rainy	1.060062	8
R	41.75	58.7	11.8215	Aug	rainy	0.981912	10
NR	41.3	61.2	5.6055	Aug	rainy	0.92957	2
R	48.7	61.2	10.3785	Aug	rainy	1.096127	3
NR	37.68	59.8	12.21	Aug	rainy	0.869024	5
R	49.15	61.4	2.2755	Aug	rainy	1.102459	6
NR	45.42	57.3	13.4865	Aug	rainy	1.095743	6
R	41.69	58.3	6.9375	Aug	rainy	0.98759	8
R	40.62	57.3	10.656	Aug	rainy	0.979944	9
R	48.86	58.7	20.3685	Aug	rainy	1.149131	9
R	45.05	59.2	4.8285	Aug	rainy	1.050098	9
NR	33.46	56.7	13.5975	Aug	rainy	0.816214	6
NR	40.05	58.1	12.0435	Aug	rainy	0.952182	12
NR	39.73	61.3	13.875	Aug	rainy	0.892696	8
NR	41.89	59.9	8.9355	Aug	rainy	0.964421	9

Continues

Table 3. Continued.

Rep. Phase	Body mass	Forearm length	Glucose (mmol/L)	Month	Season	BCI	Hours after sunset
NR	44.94	59.4	10.0455	Aug	rainy	1.043818	10
R	37.7	59.3	2.997	Sep	dry	0.877211	2
R	38.86	61.4	1.11	Sep	dry	0.871649	2
R	35.45	57.7	4.218	Sep	dry	0.848974	5
R	35.25	58.7	8.103	Sep	dry	0.82904	5
R	38.4	58.6	1.11	Sep	dry	0.904748	7
R	47.14	61.4	9.213	Sep	dry	1.057374	7
R	40.71	59.8	7.659	Sep	dry	0.938906	9
NR	38.31	58.6	1.776	Sep	dry	0.902627	10
NR	42.97	58.9	9.2685	Sep	dry	1.00699	10
R	44.74	60.3	10.656	Sep	dry	1.022838	11
R	48.26	61.3	3.8295	Sep	dry	1.084357	11
R	31.88	56	1.11	Sep	dry	0.787917	1
R	39.41	57.9	1.11	Oct	dry	0.940376	1
R	45.38	60.2	7.437	Oct	dry	1.039285	2
R	45.71	61.4	5.0505	Oct	dry	1.025298	8
R	44.08	60.7	14.1525	Oct	dry	1.000754	9
NR	49.09	61.5	14.0415	Oct	dry	1.099227	10
R	40.62	63	6.549	Nov	dry	0.886764	4
R	40.62	63	6.549	Nov	dry	0.886764	4
NR	52.51	57	14.0415	Nov	dry	1.273812	9
NR	52.51	57	14.0415	Nov	dry	1.273812	9

Ceriello 2003). However, just like birds, bats may present high levels of glucose with no adverse effects to their body condition (Braun and Sweazea 2008). Perhaps bats have evolved mechanisms to mitigate the negative effects of high levels of glucose, like high physical activity to regulate the homeostasis, such as what was described for the nectarivore *Glossophaga soricina* (Pallas, 1766) (Kelm et al. 2008) and the frugivores *Eonycteris spelaea* (Dobson, 1871) and *Cynopterus sphinx* (Vahl, 1797) (Peng et al. 2017). However, some studies have found negative correlations between blood glucose and BCIs for frugivorous bats and positive correlations for insectivorous bats in China (Meng et al. 2016). Indeed, bats from Pteropodidae may have different regulations of glucose to compare with phyllostomids bats. Recently, Amaral et al. (2018) described that the nectarivorous bat *Glossophaga soricina* (Pallas, 1766) does not have the hability to store energy becoming more susceptible to death after 18 hours of fasting. Therefore, the increasing of body mass in *A. planirostris* is more likely due to the water present in the fruits than a proper fat storage. However, we could not find other studies that have evaluated the relationship between daily body mass and blood glucose in bats. Another explanation for the different results between this study and Meng et al. (2016) is that the BCI used by the authors was the same for all species, without any kind of validation to check whether the BCI was adequate, as suggested in the study of Labocha et al. (2014). Sometimes, researchers make arbitrary choices based only on the most used indices in literature. However, it is important to consider that a good body condition index is one that can remove the effect of the body size measure on the value of body mass (Green 2001). Le Cren's index has the advantage of being adjusted to each individual population allowing to better comparisons within populations like it was the case in our study.

Although more direct measures are more reliable to predict body condition, many scientists still use morphometric BCIs, because the other measures can be expensive or difficult to perform under field conditions (Wilder et al. 2016). Here we used blood glucose assessed by tests strips and a portable glucometer that gave us satisfactory results. The portable glucometer, and inexpensive and practical tool, has been used by many studies, including ones involving free-ranging wild animals like birds (Lieske et al. 2002, Downs et al. 2010, Lobban et al. 2010). Our results were similar to Azeredo et al. (2016) who demonstrated a positive relation between glucose and the BCI of a neotropical passerine. We also could demonstrate that the body condition of *A. planirostris* can be evaluated by using blood glucose levels as the levels increased continuously along the night and accompanied the increase in the BCI.

In Brazil, some studies have used morphometric BCIs for bats as an attempt to assess the seasonality of their body condition, or used it to compare traits among populations of different habitats (Pereira et al. 2010, Morais et al. 2014, De Oliveira et al. 2017, Nunes et al. 2017). However, this is the first study to demonstrate the hourly variation and the relationship between

glucose and a morphometric BCI. Our findings also highlight the importance of noting the hour the individual was collected. In future studies about corporal condition in bats, as these measures in can change along the night this potential confounding effect must be considered.

It is important to emphasize that we did not test the accuracy of our portable glucometer by comparing its reading with other laboratory methods. Some studies demonstrated that portable glucometers do not have the same accuracy of laboratory methods when used for animals (Hollis et al. 2008, Acierno et al. 2012, Bennett et al. 2017). However, other studies pointed that glucometers can be reliable tools for veterinary purposes (Hackett and Mccue 2010, Katsoulos et al. 2011). We used the portable glucometer Optium Xceed® medsense that has already been validated to measure ketoacidosis in dairy cows (Voyvoda and Erdogan 2010) and glucose in horses (Peugnet et al. 2014). Yet, according to Stoot et al. (2014) review, the glucometer must be calibrated for species-specific blood physiology, because the values can vary among taxa, although it is still a suitable alternative tool to traditional laboratory devices. Despite these limitations, the positive correlation between the glucose levels and traditional morphometric BCI indicate that human portable glucometers can be used to complement morphometric measurements in order to evaluate the nutritional status of bats in the wild.

The present study showed a positive relationship between blood glucose level and body mass index that indicates that body mass is probably associated with the nutritional state of *A. planirostris*. Increase in glucose levels along the night can be explained by the efficient assimilation of nutrients presents in fruits ingested by the bats and the quick metabolism that increases the levels of glucose in blood. Some studies pointed that high active flight is the key to regulate homeostasis of bats. We cannot confirm that the gain of weight for *A. planirostris* is due to storing energy reserves or just the water present in fruits. Thus, we suggest that in further studies, it would be interesting to observe the action of other metabolites besides glucose, in order to better explore the body condition of bats

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